Keisuke KANAMORI*: **Origin and early development of apogamous embryos in the prothallia of *Dryopteris chinensis***

金森啓祐*：ミサキカグマの前葉体における無配生殖的な胚の起源と初期発達

Some previous authors reported the obligate apogamy in some ferns, without any direct evidences for the obligate apogamy (Farlow 1874, De Bary 1878). Recently, Manton (1950) and her followers found the obligate apogamy in many ferns from the cytological view point. On the other hand, there are only a few studies on the embryogeny of the obligate apogamy. In this paper the author will report the apogamous embryogeny in *Dryopteris chinensis* (Bak.) Koidzumi, on which the obligate apogamy was reported by Kurita (1961). The author is very indebted to Prof. Hirosi Ito for his kind guidance and valuable advice during the course of this study.

Materials and methods

The spores used in this study were collected from following five localities in Japan. They are: 1) Takanosuyama (Tokyo Pref.), 2) Kitakatusikagun (Saitama Pref.), 3) Kuroyama-santaki (Saitama Pref.), 4) Ryokamiyama (Saitama Pref.), and 5) Daibosatutoge (Yamanasi Pref.). The Meyer's medium solidified by 7.5 gr of agar per one liter was used for the cultivation of the prothallia. During the cultivation, the Meyer's solution was added on surface of the medium at intervals of ten days. The cultivation of the prothallia and the juvenile plants were carried out in the growth chamber kept at 25°C and illuminated continuously by the fluorescent lamps (600–1000 lux). The prothallia and the apogamous embryos were fixed by Craf I, and then dehydrated in the series of tertiary butyl alcohol and embedded in paraffin. Serial sections were cut 5μ thick and stained with Heidenhein's iron hematoxylin and fast green.

Observations

1. Initiation of the apogamous embryo. The spores germinate within a week and grow into the protonemas in 2 or 3 weeks after sowing. Then, they

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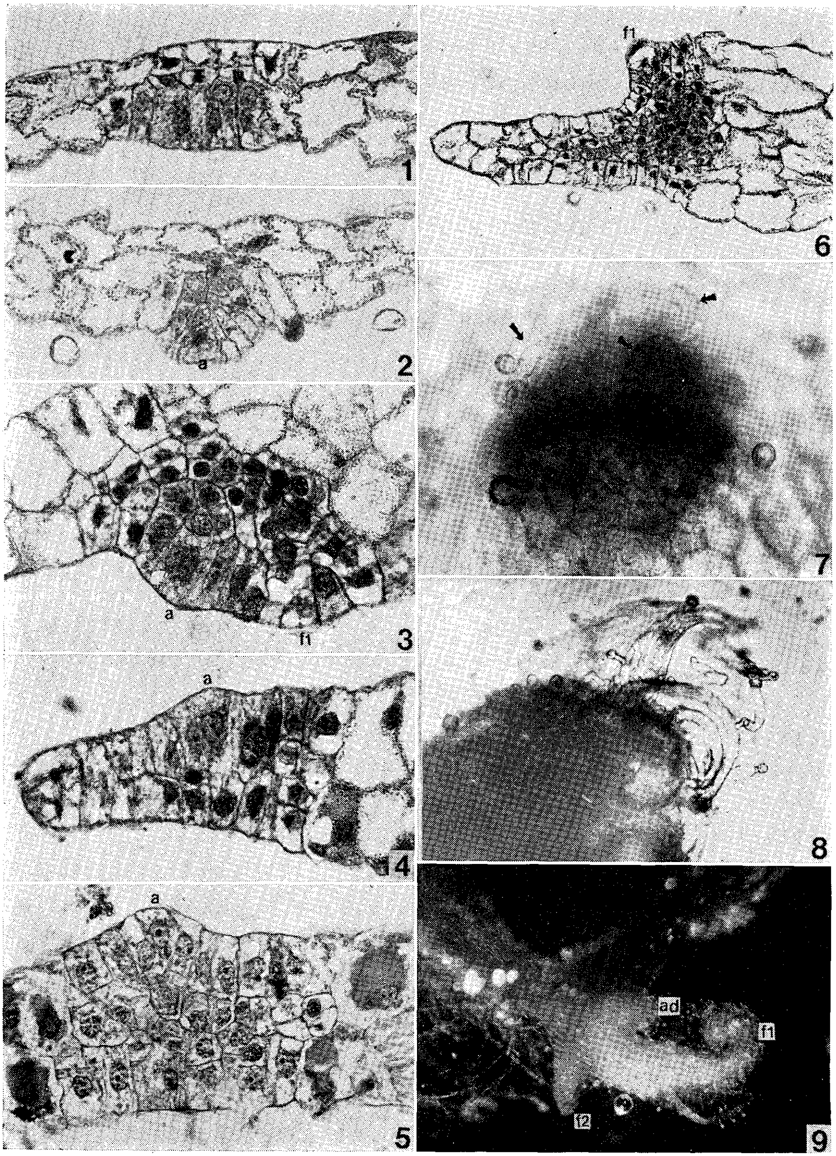

Fig. 1-9. Apogamous embryo of *Dryopteris chinensis*. Fig. 1. Cross section of cushion. Several meristematic cells are seen on the ventral side. ×220. Fig. 2. Cross section of cushion at slightly later stage of development. The embryonic mass consists of an apical cell (a). slender meristematic cells and inner small cells. ×220. Fig. 3. Longitudinal section of

develop into the heart-shaped prothallia. The cushion consisting of two layers of cells are formed almost in all the prothallia about 6 weeks after the germination. No archegonium is observed on any prothallia, but a group of small cells having dense cytoplasm is distinguished on the cushion where would produce archegonia in usual non-apogamous species. The group of cells can be seen as a dark spot on a fresh prothallium under a binocular dissecting microscope. Such a peculiar group of cells has been observed by Steil (1911, 1918, 1933) and Whittier (1964, 1965) in the prothallia of apogamous ferns. This group of cells consists of two cell layers at first, and it becomes the origin of the apogamous embryo as far as the author has observed. In regard to the development of the apogamous embryo, two different types are observed: the first type, as already observed by previous authors, is that the apogamous embyo develops on the ventral side of the prothallium, and the second type is that the embyo develops from both sides of the prothalium. These two types are tentatively called V-type and DV-type respectively and are shown diagramatically in fig. 19.

In V-type of embryo formation, cell divisions of the cells on the ventral side predominated than on the dorsal side (fig. 1). The ventral cells divide at first anticlinally, then periclinally or obliquely. During the anticlinal divisions one of these daughter cells enlarges and may be considered to become an apical cell of the embryo (fig. 2). These superficial cells and their derivatives in the cushion forms a distinct embryonic mass of tissue confirmed by large vacuolated cells of the cushion, which will develop into an embryo (figs. 2, 3). Then, this embryonic mass gradually increases in size, and its superficial region grows into a conical protrusion on the ventral surface of the prothallium, which become the apical dome of the embryo. Soon after the formation of the apical dome, another apical cell (the first leaf apical cell) is formed on its flank (fig. 3). On the other hand, the cells on the dorsal side of the prothallium do not seem to contribute to the formation of the embryo. They divide a few times and increase in size contributing to the growth of the cushion around the embryo.

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the early embryo developed from the ventral side of the prothallium (V-type). The first leaf apical cell (f_1) is differentiated on the flank of the apical dome. ×240. Fig. 4. Longitudinal section of the early embryo developed on both surfaces of the prothallium (DV-type). ×240. Fig. 5. Cross section of the early embryo (DV-type). An apical cell (a) is seen on the dorsal side. ×240. Fig. 6. Longitudinal section of stage of embryo development (DV-type). The first leaf apical cell (f_1) is seen on the dorsal side. The apical dome is not seen in this section, because it is cut somewhat obliquely. ×120. Fig. 7. Multicellular hairs on the ventral surface of the cushion (shown by the arrows). ×120. Fig. 8. Multicellular hairs and scales with prothallial papillae. ×60. Fig. 9. External view of the apogamous embryo. Apical dome (ad), first and second leaves (f_1, f_2) are seen. ×15.

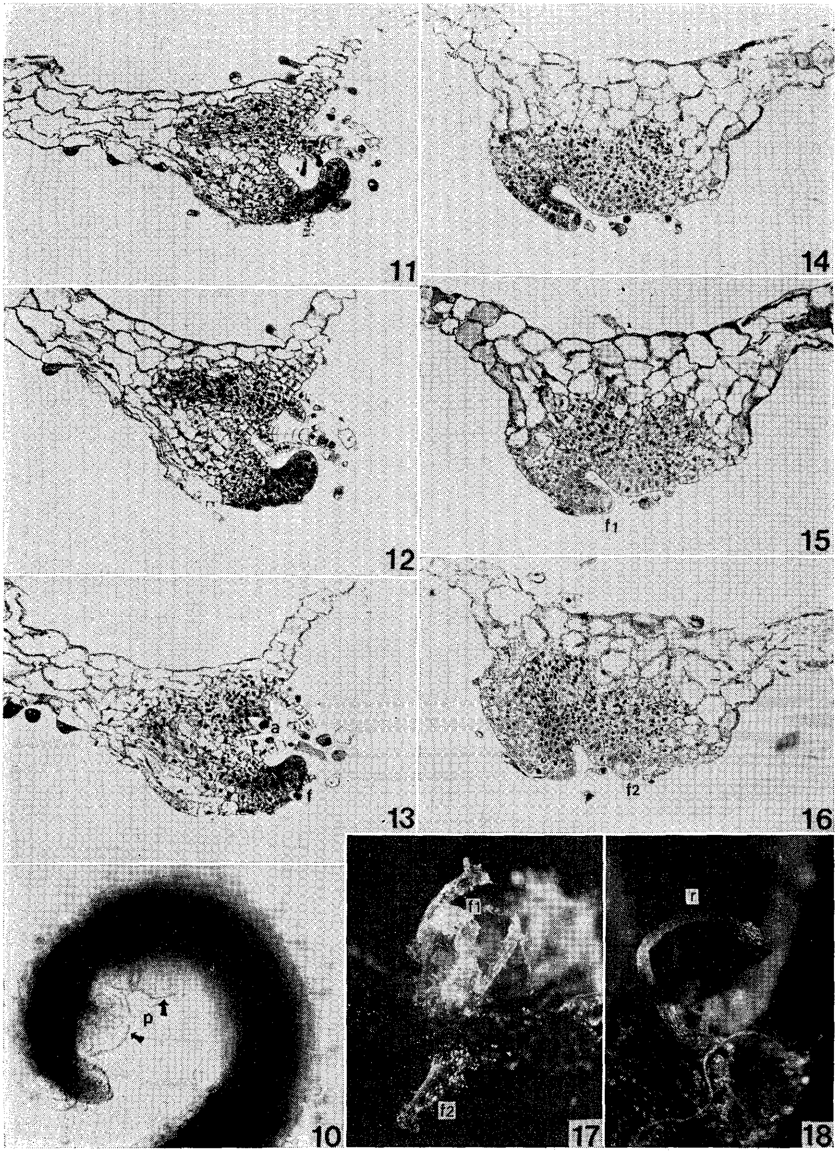
At the early stage of the embryo formation in DV-type, both dorsal and ventral cells consisting of the peculiar group in the cushion divide at first anticlinally, then periclinally or obliquely (fig. 4). So as to form the initials of the embryo composed of cells relatively small in size and rich in cytoplasm. The shoot apical cell is formed in its dorsal surface (fig. 5) in contrast to the case of the V-type. The first leaf apical cell is also differentiated on the dorsal side of the prothallium as a lateral derivative of the shoot apical cell (fig. 6).

In both types of apogamous embryo formation, a single embryo always develops at the anterior part of the cushion, though some authors have observed that apogamous embryo developed from the wing or from other parts of the prothallium (Heilbronn 1910, Steil 1918).

Previous to the external appearance of the embryo, many multicellular hairs are observed on the ventral surface of the prothallium in V-type and on the dorsal surface in DV-type. They develop into the hairs which have several papillae on tip and margin (fig. 7). Some of these hairs develop later into the colorless scales which possess many papillae (fig. 8, 9). These papillae are the same in shape as those of the prothallium and never be found in the scales of the sporophyte of this species. Therefore, it may be said that the appearance of these peculiar multicellular hairs is one of the first signs of the initiation of the apogamous embryo.

2. Development of the first leaf. At first the first leaf primordium grows straight, and then bends its apical portion toward the apical dome (figs. 9, 11–16). Soon after the beginning of the circination of the leaf primordium, the initiation of the pinna sets on (fig. 10). As far as the author has observed, the leaf does not develop into the lobed one, which is usually observed in the juvenile sporophyte developed from the fertilized egg, but it develops into a pinnate leaf with five or more pinnae. The venation is dichotomous in the pinnae. When the differentiation of the pinnae is completed, the leaf gradually uncoils. The patterns of the development of the first leaf are similar in both V- and DV-type.

3. Formation and development of the second and subsequent leaves. The second and subsequent leaves are also formed on the apical flank one by one in V-type (*fig. 16*). *These leaves develop just like the first leaf and become pinnate* ones. In DV-type, however, the second leaf arises from the ventral side of the embryo (fig. 17), while the subsequent leaves are formed on the apical flank which is located on the dorsal side of the prothallium.



Figs. 10-18. Apogamous embryo of *Dryopteris chinesis*. Fig. 10. The upper portion of the first leaf. A few pinnae (shown by the arrows) are differentiated. ×120. Figs. 11-13. Longitudinal serial sections of apogamous embyo (V-type), later stage. Tip of the first leaf primordium bends toward the apical dome. Procambial tissues are seen inner part of the embryo. 60. Figs. 14-16. Serial cross sections of apogamous embryo (V-type). ×60. Fig. 17. Later stage of the apogamous embryo (DV-type). The second leaf primordium is found on the ventral side (f 2). ×15. Fig 18. External view of the root (r) at the base of the first leaf. ×15.

4. Origination of the vascular tissues and the root. When the first leaf is initiated, some inner cells which are adjacent to the cushion cells, divide several times somewhat irregularly and become elongated (fig. 3). Most of these elongated cells are found in the innermost part of the embryo at first, and they extend later so as to form the procambial strand. The procambial cells are also found in the leaf primordium and they connect to those of the inner part of the embryo (figs. 11—16). After the completion of the first leaf, a root is differentiated endogenously at the posterior end of the embryo in relation to the base of the first leaf (fig. 18). The root apical cell which has three cutting faces divides laterally and the root primordium grows outward on the abaxial base of the first leaf. In some apogamous embryos, however, the root is not differentiated, so they do not grow beyond the stage of the completion of the first leaf and finnaly become abortive.

Discussion

Kurita (1961) reported that *D. chinensis* has gametic chromosome number 123 and this is apogamous species based on the fact that the spore number in a sporangium is 32. The author sowed the spores from five different localities three times respectively, and obtained same results in all prothallia, that is, no archegonium was formed and the apogamous embryos took place in all cultures in every time. Therefore, it may be said that this species shows surely apogamy. It has been reported by many workers that the archegonium was not found on the prothallia of some apogamous species, or if formed, it would be abortive (Farlow 1874, De Bary 1878, Steil 1918, 1919, 1933, Momose 1940, Manton 1950, Braithwaite 1964). The same observations have been obtained in the present study. Therefore, it may be considered that an abortion of the archegonium is one of the characteristics of the obligate apogamy. De Bary (1878) observed that the apogamous embryo developed on the ventral side of the prothallium. Yamanouchi (1908) reported that the apogamous embryo originated from a single large cell of the prothallium in *Nephrodium molle*. Later, Duncan (1943) confirmed De Bary's observation and thought that the apogamous embryo originates from the inner cells of the cushion in *Dryopteris pseudo-mas*. The present study shows clearly that the embryo initiates from a group of several superficial cells. This peculiar cell group appears in the cushion area at the same time of the development of the latter. The apogamous embryos developed from the several superficial cells of ventral side in many prothallia (V-type, fig. 19A). In some prothallia,

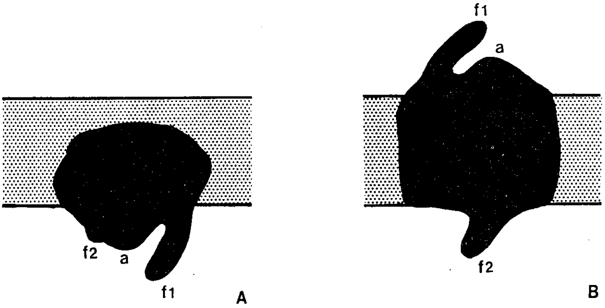


Fig. 19. Diagrams of two types of apogamous embryo formation. A. V-type; P. DV-type. Dark part is apogamous embryo and dotted part is prothallial tissue.

however, it was observed that the dorsal cells also contributed to the formation of the apogamous embryo (DV-type, fig. 19B). There are a few studies on the development of the apogamous embryo from the dorsal side of the prothallium, for example: Leitgeb (1885), Steil (1918) and Duncan (1941). They thought that such embryos might have been induced by abnormal light condition during the cultivation of the prothallia. However, in this study, the facts that both types of the embryo formation (V- and DV-type) were obtained under the same light condition may suggest some other factors rather than the light for the appearance of DV-type. In regard to the formation of the apogamous embryo in the prothallium, many previous authors reported that the leaf formed at first, then the root, and the stem finally (Steil 1918, 1919, Whittier 1965). Duncan (1943) reported that the order of the organ formation in the apogamous embyo was that the stem first, the leaf next, and the root finally. In the present study, the observations may suggest the validity of Duncan's conclusion in the sense that the stem apical cell was formed at the earliest stage of the embryo formation. The foot was not formed in this case as in other cases of the obligate apogamy.

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筆者は細胞学的に無配生殖をすることが知られているミサキカグマを用いて，その前葉体における無配生殖的な胚の起源とその初期発達の様子を調べた。胚の起源は，従来前葉体の cushion の内部の細胞や表面の1個の大きい細胞であろうと考えられていたが，この種では，cushion の表面の数個の細胞群から胚が発達することがわかった。また胚の形成に関して，胚が前葉体の腹面に発達する型（V-型）のほかに，1個の胚が前葉体の背腹両面にわたって発達する型（DV-型）が知られた。この種の無配生殖的な胚では，最初に茎，次に葉，最後に根が発達した。他の種での無配生殖的な胚と同様に，足 (foot) は観察されなかった。

□百瀬静男： **日本産シダの前葉体** B5，627 ページ，527 図，1967 年 2 月 25 日発行，東京大学出版会，6,500 円。今までシダの図鑑はいろいろ出たが，シダの前葉体だけの図鑑というものは世界でこれが初めてという，変わった内容の本である。著者は 30 何年間の前葉体専門家で，すでに 1,000 種以上の前葉体を観察記載しており，昭和12年にイタチシダの前葉体を本誌に発表して以来，ほとんど本誌がその発表機関みたいになっているので，著者の前葉体の図は読者方におなじみのところである。この本にはそのうちの代表 75 属約 330 種類を選び，未発表のものも混ぜて分類順に並べてある。ほとんど毎ページにきれいな図があり，そして詳しい記載がある。スケッチのしかた，倍率，紙，墨入れのやりかたなど，最初から現在まで一貫して変えないという著者の原図から，同一倍率に製版してあるので，全形図も部分図も皆統一されている。それゆえ各種類を比較するのに大変ぐあいがよい。今後前葉体を研究する人や胞子体の分類や系統を論ずる人にとって，十分信頼できそして特別な意見などはさまれてないすこぶる公平な図であり記載であることはまことにありがたいことである。書名の英訳は Shizuo Momose: Prothallia of the Japanese ferns (Filicales) となっている。（伊藤　洋）

□**野の花山の花 Colour Compact** 集英社　変形版 19×18 cm　青山富士夫・亘理俊次両氏のとった植物のカラー写真を中心にして構成された写真集。色彩もよいし色々珍らしい姿もみられるので植物学にもよい参考になる。ホトケノザの花，オキナグサの花柱の毛の生態，アサマフウロの花，コウリンカの花，ウメバチソウの仮おしべ，ハマウツボの生態，エンビセンノウの花などはその一例である。¥480 は安い。（前川文夫）